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Experience and Innovation

A Non-Animal Phototoxicity Test using Epidermal Tissue Models and Cytokine Endpoints. L. Pratt, J. Sallit, M. Reeder, B. Bowen, G. DEGEORGE. MB Research Labs, In Vitro Toxicology, Spinnerstown, PA 18968. E-mail: mbinfo@mbresearch.com

Abstract:

The phototoxic potential of chemicals, cosmetics, dietary supplements and pharmaceuticals are a growing concern in the consumer products and chemical industry. Animal models of phototoxicity are expensive, slow, subjective, and not amendable to high throughput. Currently in the US, there are no regulatory agency-accepted alternative or in vitro phototoxicity tests. To address the need we have conceived a high-throughput in vitro screening test for phototoxicity designated the Enhanced Phototoxicity Assay in Reconstituted Skin (EPARS). The EPARS test overcomes many of the limitations of the 3T3 NRU Phototoxicity Test; which has been validated in Europe by ECVAM: 1) EPARS is based upon a differentiated tissue model that closely parallels human skin morphology, instead of a fibroblast monolayer; 2) formulations of test articles can be topically applied instead of the often problematic solubilization of formulations into culture media; 3) the tissues are composed of human primary keratinocytes which are more relevant model than a mouse tumor cell line. In EPARS, the test substance is applied topically to the reconstituted human skin models, with and without UV irradiation. Overall, the EPARS test has proven to be an accurate and sensitive test for detecting phototoxic (photo-irritating) substances. Phototoxic effects are determined by comparing the viability of irradiated vs. non-irradiated tissues by MTT uptake. In order to increase the sensitivity and specificity of the test, we have measured the release of cytokines into the culture media via ELISA. PGE₂ release was shown to be an early predictor of the toxic effects demonstrated in the viability assay. Release of IL-1 alpha, IL-1ra, IL-8 & TNF-alpha supported the results of the cell viability. The effects of irradiation +/- chlorpromazine were further characterized by gene expression (cDNA microarray) analysis.